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Immunophenotyping with CD135 and CD117 predicts the *FLT3*, *IL-7R* and *TLX3* gene mutations in childhood T-cell acute leukemia

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ABSTRACT

With the combination of immunophenotyping and molecular tests, it is still a challenge to identify the characteristics of T cell acute lymphoblastic leukemia (T-ALL) associated with distinct outcomes. This study tests the possible correlation of cellular expression of CD135 and CD117 with somatic gene mutations in T-ALL. One hundred sixty-two samples were tested, including 143 at diagnosis, 15 from T-lymphoblastic lymphoma at relapse, and four relapse samples from sequential follow-up of T-ALL. CD135 and CD117 monoclonal antibodies were included in the T-ALL panel of flow cytometry. The percentage of cells positivity and the median fluorescence intensity were correlated with gene mutational status. *STIL-TAL1*, *TLX3*, *FLT3* and *IL7R* mutations were tested using standard techniques.

STIL-TAL1 was found in 24.8%, *TLX3* in 12%, *IL7R* in 10% and *FLT3-ITD* in 5% of cases. *FLT3* and *IL7R* mutations were mutually exclusive, as were *FLT3-ITD* and *STIL-TAL1*. Associations of $CD135^{high}$ ($p < 0.01$), $CD117^{intermediate/high}$ ($p = 0.02$) and *FLT3-ITD*, $CD117^{low}$ with $IL7R^{mutated}$ ($p < 0.01$) and $CD135^{high}$ with $TLX3^{pos}$ were observed.

We conclude that the addition of CD135 and CD117 to the diagnosis can predict molecular aberrations in T-ALL settings, mainly segregating patients with *FLT3-ITD*, who would benefit from treatment with inhibitors of tyrosine.

Abbreviations: ALL, acute lymphoblastic leukemia; APC, allophycocyanin; AML, Acute myeloid leukemia; Bcp-ALL, B-cell precursor ALL; BM, bone marrow; BFM, Berlin–Frankfurt–Munster; cy, cytoplasmatic; DS, diagnosis sample; EGIL, European group for the immunological characterization of leukemia; FITC, fluroscein isothiocyanate; GBTLI-ALL, Brazilian Group for Treatment of Childhood Leukemia; ITD, internal tandem duplication; MFI, median fluorescence intensity; M-FCM, multiparametric flow cytometry; MoAb, monoclonal antibody; mut, mutated; Neg, negative; PB, peripheral blood; PE, phycoerythrin; PerCP, peridinin-chlorophyll protein; Pos, positive; RS, relapse sample; SSC, side scatter; T-ALL, T-cell acute lymphoblastic leukemia; T-LL, T-lymphoblastic lymphoma; T-LL RSL, relapse in bone marrow of T-LL diagnosis; TCR, T-cell receptor; TKD, tyrosine kinase domain; WBC, white blood cell count; WT, wild Type.

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1. Introduction

T-cell acute lymphoblastic leukemia (T-ALL) in childhood is an aggressive malignancy characterized by high white blood cell count (WBC), mediastinal tumor and a high rate of relapses in the central nervous system, bone marrow (BM) or testicle infiltration [1]. T-ALL accounts for approximately 15% of all childhood acute lymphoblastic leukemia (ALL), and in contrast to B-cell ALL, the prognostic significance of molecular-cytogenetic abnormalities in pediatric T-ALL has not been yet elucidated [2]. Therefore, exploring the immunophenotype and molecular connections to define some T-ALL settings is one of the potential issues for clinical translation. T-ALL can be classified according to specific subgroups that are associated with the expression of specific immunophenotypic markers, reflecting maturational arrest at distinct T-cell developmental stages [2].

FMS-like kinase-3 (FLT3/CD135) and stem cell factor receptor c-Kit/CD117) are members of the class III receptor tyrosine kinase family and share the common structure of five extracellular immunoglobulin-like domains, a single transmembrane segment, a juxtamembrane domain and a split cytoplasmic domain [3]. They mutually contribute to the normal differentiation and proliferation of primitive hematopoietic cells. FLT3 and CD117 are expressed at high levels in early cell populations with lymphoid and myeloid differentiation potential [4].

Mutations in *FLT3* such as internal tandem duplication (ITDs) in the juxtamembrane domain or in the tyrosine kinase domain (TKD) result in constitutive activation of the FLT3 receptor in the absence of a ligant [4]. Both genetic abnormalities are well defined in acute myeloid leukemia (AML) and confer a poor clinical prognosis [5]. At the same time, these mutations are rarely found in childhood ALL [6–8], and the prognosis of *FLT3*-mutated patients has not yet been tested due to the low frequency of this mutation in this disease. Recently, some authors have demonstrated that immature T-ALL might benefit from receptor-kinase inhibition drug therapeutic strategies depending on the *FLT3* status [9], as in patients with B-cell precursor ALL (Bcp-ALL) with a Ph-like genome profile [10].

Because very few data are available regarding the *FLT3* mutational status and correlation with immunophenotyping in an exclusive pediatric T-ALL cohort, the present study aimed to establish the distribution frequencies of *FLT3* mutations and to test the association between *FLT3* status and CD135 and CD117 identified by flow cytometry in pediatric T-ALL.

2. Materials and methods

2.1. Patients

One hundred seventy-one unselected samples from T-cell leukemia patients (aged less than 19 years old) were included for immunophenotyping and molecular tests (shown in Fig. 1). Bone marrow (BM) aspirates and/or peripheral blood (PB) samples were sent to the Pediatric Hematology–Oncology Program, Research Centre, Instituto Nacional de Cancer, Rio de Janeiro, Brazil, for cellular and molecular diagnostic tests before treatment decisions were made. The referring physicians provided demographic and clinical information regarding age strata, gender, mediastinal mass, nervous system involvement, and white blood cell (WBC) count. A total of 166 samples were collected from 147 patients at diagnosis prior to any oncological treatment (n = 143, only once); 15 samples were obtained from T-ALL as first relapse in BM of T-lymphoblastic lymphoma diagnosis (T-LL RSL, only once), and sequential samples were collected from T-ALL patients collected at diagnosis (DS) and at first relapse in BM (RS), as shown in Fig. 1. Patients were not enrolled in clinical trials but were receiving treatments according to the Brazilian Group for Treatment of Childhood Leukemia (GBTLI-ALL 93 and 99, n = 92) or were treated according to the Berlin–Frankfurt–Munster ALL (BFM; n = 66) protocols backbone strategies [11,12].

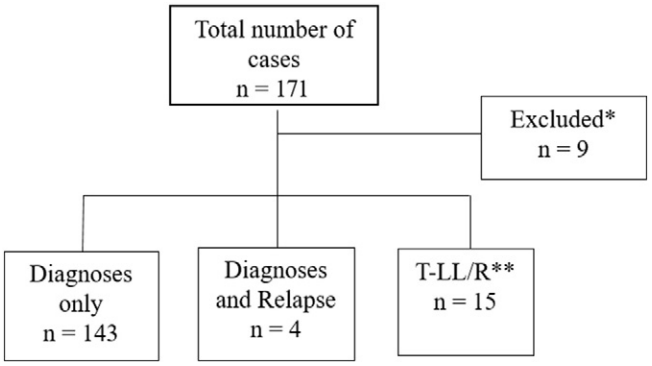


Fig. 1. The characterization of the T-ALL cases and samples included in the study. *There were 9 out of 171 (5.2%) cases excluded due to lack of material for all analysis, low percentage of blast cells or acute leukemia with mixed phenotype; 8 samples are sequential from the diagnosis (n = 4) and first relapse (n = 4); **15 cases were T-cell lymphoblastic lymphoma (T-LL) at first relapse in bone marrow/peripheral blood as T-ALL.

2.2. T-ALL diagnosis by flow cytometry

Immunophenotyping was performed by multiparametric flow cytometry (M-FCM). In the first step, cytoplasmic (cy) antigens were tested with a-MPO, CD13, IgM, CD79a, TdT, CD3, and CD22. Positive cyCD3 and negative aMPO, CD22 and CD79a defined the T-cell leukemia; then, a panel of T-cell-associated membrane antigen monoclonal antibodies were applied for cell differentiation level and anomalous expression in T-ALL, including cyCD3/CD7, CD2, CD5, CD1a, CD4, CD8, mCD3, TCR αβ, TCR γδ, HLA-DR, CD34, CD10, CD13, CD33, CD11b, CD15, CD16, CD56, and TdT in four or six colors. FLT3 receptor (CD135) (4G8) and c-KIT ligand (CD117) (104D2) conjugated to phycoerythrin (PE) were analyzed using triple or quadruple staining with fluorescein isothiocyanate (FITC), pyridine-chlorophyll protein (PerCP) and allophycocyanin (APC) in combination with anti-CD34, anti-CD7, anti-CD2, anti-CD5 and anti-CD45 monoclonal antibodies.

FACS Calibur and Canto Flow cytometers (Becton, Dickinson and Company, CA, USA) were used for the acquisition (approximately 20.000 events) in the case of blasts cells ($CD45^{low/intermediate}$). The normalization of median fluorescence intensity (MFI) of CD117 and CD135 was defined as a ratio (MFI-r) between the MFI values of CD117 and CD135 on leukemic blasts and on a population of cells non-specifically stained with IgG of the same isotype and conjugated to the same fluorochrome. Summit 5.2 software (Dako, Glostrup, Denmark) was used for analysis and determination of the median fluorescence intensity (MFI) and the percentage of cells positive to CD135 and CD117. The analysis was initially performed using side scatter versus CD45 parameters. A sample was considered to have positive markers when at least 20% of the blasts had a specific immunophenotypic marker in a live gate $CD45^{low/intermediate}$. Immunological classification of T-ALL subsets was based on previously published criteria [13–15]. The criterion for cellular antigen expression for CD135 and CD117 was determined as high, medium or low cellular expression, which was defined according to the percentile values of MIF-r. Cases with values of MFI-r below the median were considered to have low expression, cases with a value above the median and below 75th percentile were considered to have medium expression and cases with values above 75th were considered to have high expression.

The presence or absence of blast cells in the samples was reviewed before any molecular analysis was performed. To diagnose leukemia, a threshold of >20% of blast cells in the BM was used as a lower limit for further analysis in T-ALL. In the PB, a clinically high WBC count or a blast percent ≥25% were considered suitable for any test.

2.3. Molecular Analysis

DS, T-LL RSL and RS were first subjected to total RNA extraction using TRIzol (Life Technologies, Grand Island, NY, USA) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized by using 2 μg of total RNA with the First-Strand cDNA Synthesis Kit™ (Amersham Pharmacia Biotech, Amersham Biosciences UK Limited, Little Chalfont/UK). The integrity of the RNA and cDNA was examined by amplifying a fragment of the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) constitutive gene according to reported conditions [16]. Genomic DNA from the same leukemic cell samples were also obtained using a QIAamp® DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany) as recommended by the protocol.

2.4. Identification of STIL-TAL1 fusion gene and TLX3 transcript

The *STIL-TAL1* fusion gene status and the presence of the *TLX3* (*HOX11L2*) transcript were assessed by reverse transcriptase–polymerase chain reaction (RT–PCR) technique using primers and conditions as previously described [16].

2.5. Mutational analysis in the genes FLT3 and IL7R

FLT3-D835 mutations in exon 17 were detected by a restriction fragment length mediated PCR assay (RFLP). To detect *FLT3* mutations in the juxtamembrane domain, the (*FLT3*-ITD) region spanning exons 11 and 12 was amplified using the primers, PCR reactions and cycle conditions described previously [17,18].

IL7R mutations (exon 6) were detected by direct sequencing using the primers, PCR amplification conditions previously described for gene region [19].

For all sequencing analyses, the PCR products were purified using the GFX PCR DNA and a Gel Band Purification Kit (GE Healthcare, Amersham, UK) and sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA) with a 3500 genetic analyzer (Applied Biosystems). The analyses were performed with BioEdit 7.0.9 software, comparing electropherograms with the reference sequence accessed from the National Center for Biotechnology Information (NCBI) for *IL7R* (*IL7R*_NM_002185; NP-002176; NG-009567).

2.6. FLT3 real-time quantitative-PCR analysis

Real-time quantitative-PCR (qRT-PCR) was performed to determine the expression levels of *FLT3* transcription. qRT-PCR was conducted using a ViiA™7 Real-Time PCR System (Applied Biosystems, CA, USA). Reactions were performed in duplicate and were assessed using SYBR Green Go Taq® qPCR Master Mix (Promega, Madison, USA). The relative target mRNA expression was determined using the comparative Δcycle threshold (ΔCT) method. *GAPDH* was used as an internal control. Threshold values were calculated and normalized with *GAPDH*, and ΔCT was used to quantify *FLT3* gene expression, as analyzed using Expression Suite Software Version 1.0.4 (Life Technologies, CA, USA). The primer pairs used for *FLT3* were Forward AGCATCCCAGTCAATCAG and Reverse CTGGCTGGTGCTTATGA (5′ to 3′).

2.7. Statistical analysis

To compare the distribution of categorical variables, the Chi-square test was used, and Fisher's exact test (2-tailed) was used when the expected count in at least one cell of the table was less than five. Cases were grouped as T-ALL prior to any treatment (DS) or T-ALL as first relapse of prior T-LL diagnosis (T-LL, RSL). Cases were grouped as *FLT3-WT* or *FLT3-ITD*, and both groups were analyzed by age range, gender, and immune-molecular and clinical features to compare for significant differences. The Mann–Whitney U test was used to test the differences in continuous variables (percentage-positivity and MIF-r of CD135 and CD117) in models that included *FLT3*, *IL7R*, *STIL-TAL1* and *TLX3* status. Receiver operating characteristic (ROC) curves were generated to determine the likelihood expression of CD117 and CD135 to predict genotype status. The ROC curves are tools to test accuracy, sensitivity and specificity of association between cell markers. The Spearman rank correlation coefficient was calculated for both cellular (CD117 and CD135) and gene expression (*FLT3*). All tests were performed using PRISM software (PRISM, Graphpad, La Jolla, CA, USA). p-Values < 0.05 were considered statistically significant.

2.8. Ethical aspects

Patients were cared for in accordance with the Declaration of Helsinki ethical standards. The Ethical and Scientific Committees of the Instituto Nacional de Cancer, Rio de Janeiro, Brazil, approved the study (CEP/INCA#117/12; CEP-CONEP: PB #888.277).

3. Results

The demographics and main clinical and laboratorial features of the 162 T-ALL/LL patients included in this study are shown in Table 1. To examine possible bias introduced by selecting variables regarding T-ALL (*de novo*) and T-ALL secondary to T-LL, the demographics, clinical, and laboratorial molecular characteristic were tested for homogeneity. Almost all variables were similar, including the male: female ratio (3:1) in both groups; the mediastinal mass and WBC were higher in T-ALL ($p = 0.03$ and 0.001, respectively). T-cortical was the more frequent

Table 1
Demography and clinical features of T-ALL at diagnosis and lymphoblastic lymphoma with bone marrow relapse.

	N (%)[a]	T-ALL (DS)	T-LL RSL	*p*
Age				
<10 year	85 (52.5)	81 (55.1)	4 (26.7)	0.05
10–18 years	77 (47.5)	66 (44.9)	11 (73.3)	
Gender				0.5
Male	121 (74.7)	111 (75.5)	10 (66.7)	
Female	41 (25.3)	36 (24.5)	5 (33.3)	
Skin color				0.83
White	66 (40.7)	60 (40.8)	6 (40	
Non-White	96 (59.3)	87(59.2)	9 (60)	
WBC ($\times 10^9$/L)				0.03
<50	51 (31.5)	42 (28.6)	9 (60)	
≥50 < 100	29 (17.9)	26 (17.7)	3 (20)	
≥100	82 (50.6)	79 (53.7)	3 (20)	
Mediastinal mass				0.01
yes	58 (35.8)	57 (38.8)	1 (6.6)	
no	104 (64.2)	90 (61.2)	14 (93.4)	
STIL-TAL1				0.99
positive	34 (26.4)	31 (26.3)	3 (27.3)	
negative	95 (73.6)	87 (73.7)	8 (72.7)	
TLX3				0.36
positive	16 (11.4)	16 (12.5)	0	
negative	124 (88.6)	112 (87.5)	12 (100)	
FLT3				0.9
ITD	8 (4.9)	7 (4.8)	1 (6.6)	
Wild-type	154 (95.1)	140 (95.2)	14 (93.4)	
IL7R				0.6
Mutated	9 (5.8)	9 (6.3%)	0	
Wild-type	147 (94.2)	135 (93.7)	12 (100)	
EGIL (WHO)[b]				0.2
ETP-ALL	12 (8.3)	11(8.3)	1 (8.3)	
T-II (Pre-T)	32 (22.1)	30 (22.6)	2 (16.7)	
T-III (T-cortical)	66 (45.5)	63 (47.4)	3 (25)	0.057
T-IV (T-mature)	35 (24.1)	29 (21.8)	6 (50)	
Total	162 (100)	147 (90.7)	15 (9.3)	

T-ALL, T cell acute leukemia at diagnosis; Ds, diagnostic sample; T-LL RSL, lymphoblastic lymphoma with bone marrow relapse.
All cases classified was T-I (pro-T) fill criteria to early T cell precursor acute lymphoblastic leukemia (ETP-ALL).
[a] N, Total number of cases.
[b] EGIL classification was not applied in 17 cases due to lack of mCD3 and/or CD1a tests.

subtype (45.5%). Furthermore, the sample consisted of 8.3% early T cell precursor ALL (ETP-ALL), 22.1% pre-T and 24.1% T mature subtypes. The prevalences of *STIL-TAL1*, *TLX3* and *IL7R* were 26.4%, 11.4% and 5.8%, respectively. There were eight cases with *FLT3-ITD* that represented 4.9% of the whole cohort. In addition, the *FLT3-D835* mutation was not observed, and the *FLT3-ITD* mutations and *IL-7R* mutations were mutually exclusive, as were the *FLT3-ITD* and *STIL-TAL1* mutations. The main characteristics of T-ALL/LL patients according to *FLT3* status are shown in Table A.1 in the appendix. There were no differences in demographics (sex, age at the diagnosis) or clinical variables (EGIL/WHO immunophenotyping, mediastinal mass, high white blood cell count or SNC involvement) according to *FLT3* status.

The T-ALL/LL subtypes determined by immunophenotyping profile and *FLT3* status are shown in Table A.2. HLA-Dr^{pos}, CD34^{pos} and CD10^{pos} were found in 21.6%, 38.6% and 42.8% of cases, respectively. Myeloid antigens (CD11b^{pos}, CD117^{pos}, CD33/CD13^{pos}) were also found in 18.2%, 20.2% and 30.5% of cases, respectively. CD135 was positive in 11.4% (10/88) of cases tested (three ETP-ALL, two pre-T, two T-cortical and three T-mature ALL). The distribution of different immunophenotypes of T-ALL/LL sub-sets was similar between patients with or without *FLT3-ITD* mutations. However, the majority of cases with *FLT3-ITD* (62.5%) were CD2/CD5^{pos} and CD4/CD8^{neg}. CD1a^{pos} was expressed at low levels (≥20% and <50% positivity blasts cell) in three of eight patients with *FLT3-ITD.* Expression of mCD3^{pos} was observed in very few cases.

To test whether cellular expression of CD135 and CD117 identified by M-FCM would predict somatic mutations, the sensitivity and specificity of CD117 and CD135 were then evaluated by MIF-r and the percentage of positive cells. The median expression levels of CD117 and CD135 were heterogeneous in the whole cohort (MIF-r, 1.9 (0.5–82), 5% (0–96%) and MIF-r 1.8 (0.5–22.3), 3% (0–97%), respectively) and were taken as an example case, as shown in Fig. 2.

However, CD117 and CD135 MIF-r and the positivity changes in the median values varied according to gene mutations. Significant differences in the cellular expression levels of CD117, CD135 were observed, as shown in Fig. 3. CD135 expression was higher in *FLT3-ITD* than in *FLT3-WT* ($p < 0.01$) or the *TLX3^{pos}* group ($p = 0.046$), whereas no differences in CD135 and CD117 expression were observed in *STIL-TAL1* cases. The lowest expression of CD117 was found in T-ALL with *IL7R* mutated compared with *IL7R-WT* ($p < 0.01$).

The cellular expression variability of CD117 and CD135 according to MIF-r percentile values and *FLT3*, *TLX3* and *ILR* status are shown in Table 2. Fifty-four percent of *TLX3^{pos}* cases and 83.3% of *FLT3-ITD* cases were associated with high CD135 MFI-r ($p < 0.01$). Regarding the expression of CD117 and *FLT3-ITD*, 50% of cases were associated with intermediate MFI-r and another 50% were associated with high MFI-r ($p = 0.03$). Conversely, all cases with *IL7R* mutated were associated with low MFI-r ($p < 0.01$) (Table 2).

The ability of CD117 and CD135 expression variability to predict molecular aberrations was tested after the adequate cutoff ratio was established in order to achieve the appropriate sensitivity and specificity (shown in appendix Table A.3). The accuracy values to predict *FLT3-ITD* were better for CD135 and CD117 MIF-r (0.86 and 0.79, respectively) than for the percentage of positivity cells (0.77 and 0.74, respectively). In addition, for *IL7R* mutation prediction, taking into account the expression of CD117 resulted in equal accuracy value for both percentage and MFI-r (0.85).

Then, the *FLT3* transcript expression was quantified by qRT-PCR in order to compare with the CD117 and CD135 cellular profiles determined by M-FCM. Thirty samples with CD117 and CD135 cellular profiles determined by M-FCM in which biological material was still available were analyzed (Fig. 4). Quantitative qRT-PCR assays revealed that 5T-ALL cases had high *FLT3* expression. *FLT3-ITD* expression was 1.55-fold higher than *FLT3-WT* expression ($p = 0.025$). The correlation of *FLT3* transcription with CD135 and CD117 MFI-r levels was tested, and positive correlations were found with both markers ($p = 0.03$; $p = 0.002$).

Finally, a summary of the demography and laboratorial findings of all patients with *FLT3-ITD* is shown in Table A.4 in the appendix. Two cases (#5; #8) were classified as ALL-ETP according to the immunophenotyping profile. In both cases, the status of *FLT3* changed during patient follow-up. The sequential samples (DS and RS) demonstrated that in one case (#5), heterozygous *FLT3-ITD* mutation was present in the DS sample and was not present in the RS sample, while in case

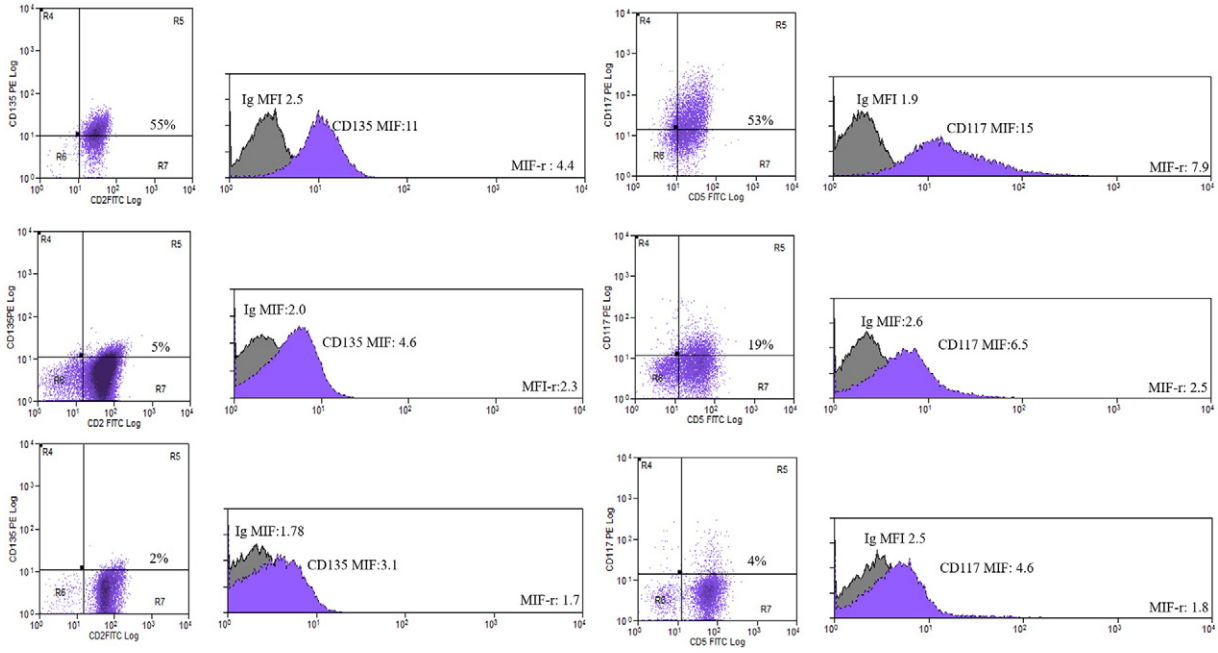
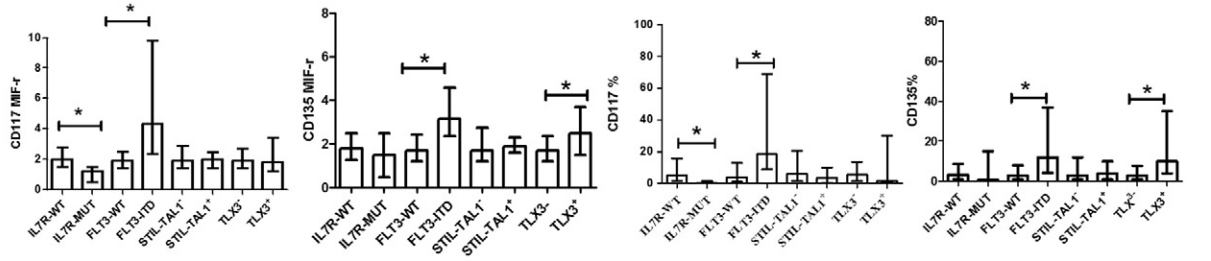


Fig. 2. Cellular expression of CD135 and CD117 antigens in blast cells analyzed by M-FCM. Cells positivity in dot plot and ratio median fluorescence intensity (MFI-r) in histograms. Non-specific IgG, CD135 and CD117 were used to define the MFI-r normalization. A) CD135 and CD117 high expression; B) CD135 and CD117 intermediate expression; C) CD135 and CD117 low expression.



	IL7RWT	IL7Rmut	p	FLT 3WT	FLT3ITD	p	STIL -TAL1 -	STIL -TAL +	p	TLX3 -	TLX3 +	p
CD135 (n=88)												
MFI -r (min -max)	1.8 (0.7 -22.3)	1.5 (0.5 -3.2)	0.18	1.7 (0.5 -22.3)	3.2 (1.9 -6.4)	*<0.01*	1.7 (0.7 -22.3)	1.9 (0.5 -3.3)	0.57	1.7 (0.5 -6.4)	2.5 (1.2 -22.3)	*0.046*
% (min -max)	3.3 (0-97)	0.8 (0-17)	0.085	3.0 (0.0 -97)	25 (2-42)	*0.02*	3.0 (0.0 -97)	4.0 (0.0 -19)	0.96	3.0 (0.0 -42)	10.0 (0.8 -97)	*0.01*
CD117 (n=94)												
MFI -r (min -max)	2 (0.7 -82)	1.2 (0.5 -1.8)	*0.002*	1.9 (0.5 -82)	4.3 (2.0 -20.7)	*0.01*	1.9 (0.7 -82)	2.0 (0.5 -30. 8)	0.92	1.9 (0.5 -82)	1.8 (0.7 -16.7)	0.72
% (min -max)	5.0 (0-96)	0.3 (0-1.7)	*0.002*	4 (0-96)	18.5 (3-90)	*0.03*	6 (0-96)	3.5 (0-92)	0.27	5.5 (0-95)	1.7 (0-96)	0.58

Fig. 3. Differences of cellular expression of CD117 and CD135 according to molecular alteration. *p < 0.05; interquartile range of MIF-r (ratio of median fluorescence intensity); positive cells (%) of CD117 and CD135. In tables the median, minimum and maximum value of MFI-r and positive cells (%) according to gene status. Mut, mutated; WT, wild-type, ITD, internal tandem duplication.

#8, heterozygous *FLT3-ITD* mutation was present in the DS and changed to the *FLT3-ITD* homozygous pattern in the RS (17 months after DS). Both patients died during relapse.

4. Discussion

The application of M-FCM is widely accepted as a frontline test for the identification of ALL subtypes for risk stratification and treatment. However, the correlation of antigen expression and somatic genotyping of T-ALL has not been explored as in Bcp-ALL, in which the antigen expression patterns reflecting the genotype are associated with molecular-cytogenetic abnormalities that predict prognosis [20,21]. Therefore, efforts have been made to achieve sensitivity and specificity values using M-FCM to identify T-ALL subsets associated with somatic genetic aberrations [22,23].

In the present study, the accuracy of CD135 (FLT3) and CD117 (c-KIT) were explored in a large cohort of T-ALL samples from Brazil, seeking possible correlations with T-ALL-specific gene aberrations such as *STIL-TAL*, *TLX3* and *IL7R* mutations. The frequency of the *FLT3* mutation was low (5%) compared with other molecular aberrations, and only *FLT3-ITD* was identified, similar to previous studies [6,8]. *STIL-TAL*, *TLX3* and *IL7R* were found in according to previous reports [15,17], and *FLT3-ITD* and *IL7R* mutations were mutually exclusive, as were *FLT3-ITD* and *STIL-TAL1*.

Although the CD135 antigen is recognized as the FLT3 ligand to the *FLT3* receptor–signaling pathway, it is rarely tested in acute leukemia [24]. A few studies have found that the CD135 antigen is highly expressed in Bcp-ALL, B-cell line and acute myeloid leukemia (AML) and in a minor percentage in T-cell ALL [25]. The cellular expression of CD135 was found in 11.4% of the cases tested, and the frequency was lower than that of CD117 (20%). However, the correlation test indicates that a high level of combining cell markers is a good predictor of *TLX3*, *FLT3-ITD* and *IL7R* gene mutations. The cellular expression in terms of positivity and MFI-r of CD117 and CD135 was greater in cases with *FLT3-ITD*, although *FLT3-WT* cases were also found with CD135 and CD117 antigens. These findings were confirmed in the positive correlation of high levels of *FLT3* transcripts in cases of *FLT3-ITD* and *FLT3-WT*. Furthermore, we observed that CD135 antigen expression in blast cells was higher in cases with $TLX3^{pos}$, in contrast to $STIL\text{-}TAL1^{pos}$ in T-ALL. These findings are in agreement with those of Lhermitte et al. (2013) [9], who suggested that FLT3 protein expression can be ectopic and overrepresented in subsets of T-ALLs and that expression of *FLT3* was higher in oncogenic groups associated with TCRγδ lineage orientation, such as *TLX3*, and lower in oncogenic groups with αβ lineage orientation, such as *STIL-TAL* [9,26].

Recently, CD117 expression was found in a T-ALL subset known as early T-cell precursor acute lymphoblastic leukemia (ETP-ALL), which is characterized by less than 5% CD1a and CD8, $CD5^{weak}$ with less than 75% blasts and the presence of stem cell (CD34, HLA-DR) or myeloid markers (CD117, CD13, CD33, CD11b, CD15, CD65) [22]. ETP-ALL demonstrates unfavorable outcomes in adults and children and is associated with a high frequency of *FLT3* mutation [27,28]. However, even more

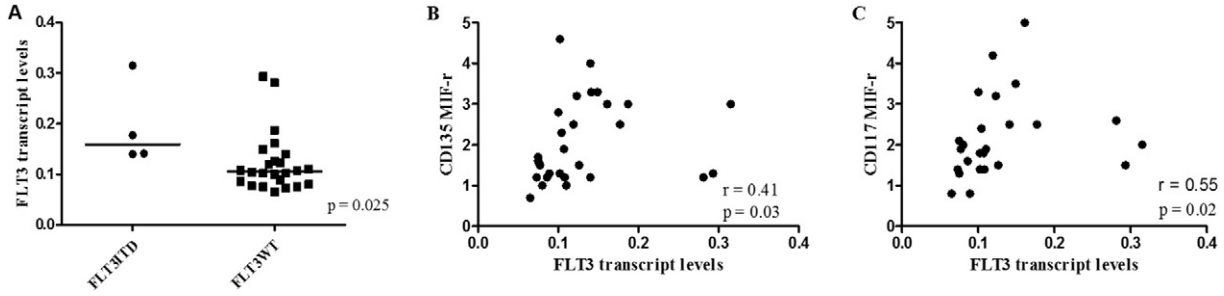


Fig. 4. Comparisons between *FLT3* status and CD135 and CD117 cellular expression and *FLT3* transcript. A) Differences of *FLT3* transcript expression according to *FLT3* mutation status, calculated by Mann–Whitney test; the median expression is shown by horizontal bar; B) correlation between CD135 cellular expression and *FLT3* transcript expression levels. C) correlation between CD117 cellular expression and *FLT3* transcript expression. r, Spearman coefficient; p value.

Table 2
Expression variability between molecular alterations according to MIF-r percentile values of CD117 and CD135.

	Low	Intermediate	High	p
CD135 MIF-r	(<1.8)	(≥1.8 < 2.5)	(≥2.5)	
FLT3-ITD (n = 6)	0	1 (16.7)	5 (83.3)	<0.01
FLT3-WT (n = 82)	42 (51.2)	20 (24.4)	20 (24.4)	
TLX3^{+} (n = 11)	3 (27.3)	2 (18.2)	6 (54.5)	0.13
TLX3^{-} (n = 69)	36 (52.2)	17 (24.6)	16 (23.2)	
CD117 MIF-r	(<1.9)	(≥1.9 < 2.7)	(≥ 2.7)	
FLT3-ITD (n = 6)	0	3 (50)	3 (50)	0.03
FLT3-WT (n = 88)	43 (48.8)	24 (27.3)	21 (23.9)	
IL7R mutated (n = 7)	7 (100)	0	0	<0.01
IL7R-WT (n = 86)	35 (40.7)	27 (31.4)	24 (27.9)	

Abbreviations: ratio median fluorescence intensity (MFI-r); WT, wild-type; ITD, Internal tandem duplication.

importantly, CD117 alone does not predict *FLT3* mutation in T-ALL cases [6,24,29–31]. In the majority of the studies, mixed-lineage T/myeloid was involved or the studies did not include pediatric cohorts exclusively.

In our study, two cases with *FLT3-ITD* fulfilled the criteria for ETP-ALL, but *FLT3-ITD* was not restricted to more immature T-ALL; although most cases with *FLT3-ITD* were CD4^{neg}/CD8^{neg}, we found cases CD4^{pos}/CD8^{neg} and CD4$^{pos/}$CD8^{pos}. These immunophenotypic features were also described in previous studies [6,9]. Interestingly, TCRγδ was detected in two cases with *FLT3-ITD*, and these data suggest that *FLT3-ITD* is restricted to the TCRγδ lineage and is not found in the αβ lineage [9].

In T-ALL, CD117 expression was found in 9 to 28% of cases [32–34]. No correlation between the expression of CD117 and *STIL-TAL1*^{pos} or *TLX3*^{pos} was observed. The expression of CD117 was lowest in cases of *IL7R*-mutations. Mutations in *IL7R* appear to influence the expression of CD117 in accordance with the already described cross talk between these receptors [35].

Another point that should be raised is the different outcomes of two patients with *FLT3-ITD*; one relapsed 17 months after initial diagnosis with no wild-type *FLT3* allele and only mutated alleles, probably due to loss of heterozygosity. Conversely, another patient showed no *FLT3/ITD* mutation at relapse, consistent with suggestions by Van Vlierberghe et al. (2005) that the loss of the mutated allele occurred during therapy or that the clone positive for *FLT3-ITD* was eliminated during chemotherapy with a relapse from an *FLT3 WT* parental clone [6].

5. Conclusion

FLT-ITD in children with T-ALL can be predicted with the addition of CD135 and CD117 in the M-FCM panel of monoclonal antibodies, taking into account that MFI-r values are better than the percentage of positivity. Although this mutation occurs at a low frequency, the identification of *FLT3-ITD* can segregate patients who would benefit from treatment with inhibitors of tyrosine kinase.

Conflict of interests

The authors have no competing interests.

Authors' contributions

Eugenia Terra Granado, Francianne Gomes Andrade, Carolina Zampier, Camila Andrade, Elda Pereira Noronha and Maria S. Pombo-de-Oliveira wrote the manuscript. EN and ETG performed immunophenotype. EN, FGC, CZ and CA performed cytogenetic and molecular assays. MSPO contributed to the conception of the study, writings and critical analysis of the data. All co-authors from the BSGCL contributed with clinical and demographical data.

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Appendix A. Supplementary data

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References

[1] C.H. Pui, M.V. Relling, J.R. Downing, Acute lymphoblastic leukemia, N. Engl. J. Med. 350 (2004) 1535–1548.
[2] J.P. Meijerink, Genetic rearrangements in relation to immunophenotype and outcome in T-cell acute lymphoblastic leukaemia, Best Pract. Res. Clin. Haematol. 23 (2010) 307–318.
[3] K. Masson, L. Ronnstrand, Oncogenic signaling from the hematopoietic growth factor receptors c-Kit and Flt3, Cell. Signal. 21 (2009) 1717–1726.
[4] D.G. Gilliland, J.D. Griffin, The roles of FLT3 in hematopoiesis and leukemia, Blood 100 (2002) 1532–1542.
[5] A. Staffas, M. Kanduri, R. Hovland, R. Rosenquist, H.B. Ommen, J. Abrahamsson, E. Forestier, K. Jahnukainen, O.G. Jonsson, B. Zeller, J. Palle, G. Lonnerholm, H. Hasle, L. Palmqvist, H. Ehrencrona, Presence of FLT3-ITD and high BAALC expression are independent prognostic markers in childhood acute myeloid leukemia, Blood 118 (2011) 5905–5913.
[6] P. Van Vlierberghe, J.P. Meijerink, R.W. Stam, W. van der Smissen, E.R. van Wering, H.B. Beverloo, R. Pieters, Activating FLT3 mutations in CD4^{+}/CD8^{-} pediatric T-cell acute lymphoblastic leukemias, Blood 106 (2005) 4414–4415.
[7] A. Andersson, K. Paulsson, H. Lilljebjorn, C. Lassen, B. Strombeck, J. Heldrup, M. Behrendtz, B. Johansson, T. Fioretos, FLT3 mutations in a 10 year consecutive series of 177 childhood acute leukemias and their impact on global gene expression patterns, Genes Chromosom. Cancer 47 (2008) 64–70.
[8] M.D. Kraszewska, M. Dawidowska, M. Kosmalska, L. Sedek, W. Grzeszczak, J.R. Kowalczyk, T. Szczepanski, M. Witt, BCL11B, FLT3, NOTCH1 and FBXW7 mutation status in T-cell acute lymphoblastic leukemia patients, Blood Cells Mol. Dis. 50 (2013) 33–38.
[9] L. Lhermitte, R. Ben Abdelali, P. Villarese, N. Bedjaoui, V. Guillemot, A. Trinquand, M. Libura, A.S. Bedin, A. Petit, H. Dombret, G. Leverger, N. Ifrah, O. Hermine, E. Macintyre, V. Asnafi, Receptor kinase profiles identify a rationale for multitarget kinase inhibition in immature T-ALL, Leukemia 27 (2013) 305–314.
[10] K.G. Roberts, Y. Li, D. Payne-Turner, R.C. Harvey, Y.L. Yang, D. Pei, K. McCastlain, L. Ding, C. Lu, G. Song, J. Ma, J. Becksfort, M. Rusch, S.C. Chen, J. Easton, J. Cheng, K. Boggs, N. Santiago-Morales, I. Iacobucci, R.S. Fulton, J. Wen, M. Valentine, C. Cheng, S.W. Paugh, M. Devidas, I.M. Chen, S. Reshmi, A. Smith, E. Hedlund, P. Gupta, P. Nagahawatte, G. Wu, X. Chen, D. Yergeau, B. Vadodaria, H. Mulder, N.J. Winick, E.C. Larsen, W.L. Carroll, N.A. Heerema, A.J. Carroll, G. Grayson, S.K. Tasian, A.S. Moore, F. Keller, M. Frei-Jones, J.A. Whitlock, E.A. Raetz, D.L. White, T.P. Hughes, J.M. Guidry Auvil, M.A. Smith, G. Marcucci, C.D. Bloomfield, K. Mrozek, J. Kohlschmidt, W. Stock, S.M. Kornblau, M. Konopleva, E. Paietta, C.H. Pui, S. Jeha, M.V. Relling, W.E. Evans, D.S. Gerhard, J.M. Gastier-Foster, E. Mardis, R.K. Wilson, M.L. Loh, J.R. Downing, S.P. Hunger, C.L. Willman, J. Zhang, C.G. Mullighan, Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia, N. Engl. J. Med. 371 (2014) 1005–1015.
[11] A. Moricke, A. Reiter, M. Zimmermann, H. Gadner, M. Stanulla, M. Dordelmann, L. Loning, R. Beier, W.D. Ludwig, R. Ratei, J. Harbott, J. Boos, G. Mann, F. Niggli, A. Feldges, G. Henze, K. Welte, J.D. Beck, T. Klingebiel, C. Niemeyer, F. Zintl, U. Bode, C. Urban, H. Wehinger, D. Niethammer, H. Riehm, M. Schrappe, Risk-adjusted therapy of acute lymphoblastic leukemia can decrease treatment burden and improve survival: treatment results of 2169 unselected pediatric and adolescent patients enrolled in the trial ALL-BFM 95, Blood 111 (2008) 4477–4489.
[12] S.R. Brandalise, V.R. Pinheiro, S.S. Aguiar, E.I. Matsuda, R. Otubo, J.A. Yunes, W.V. Pereira, E.G. Carvalho, L.M. Cristofani, M.S. Souza, M.L. Lee, J.A. Dobbin, M.S. Pombo-de-Oliveira, L.F. Lopes, K.N. Melnikoff, A.L. Brunetto, L.G. Tone, C.A. Scrideli, V.L. Morais, M.B. Viana, Benefits of the intermittent use of 6-mercaptopurine and methotrexate in maintenance treatment for low-risk acute lymphoblastic leukemia in children: randomized trial from the Brazilian Childhood Cooperative Group–protocol ALL-99, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 28 (2010) 1911–1918.
[13] M.C. Bene, G. Castoldi, W. Knapp, W.D. Ludwig, E. Matutes, A. Orfao, M.B. van't Veer, Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL), Leukemia 9 (1995) 1783–1786.

[14] W. Gorczyca, J. Weisberger, Z. Liu, P. Tsang, M. Hossein, C.D. Wu, H. Dong, J.Y. Wong, S. Tugulea, S. Dee, M.R. Melamed, Z. Darzynkiewicz, An approach to diagnosis of T-cell lymphoproliferative disorders by flow cytometry, Cytometry 50 (2002) 177–190.
[15] J.K.C.C., M.J. Borowitz, T Lymphoblastic Leukaemia/Lymphoma, Fourth Edition ed. International Agency for Resarch on Cancer, Lyon:France, 2008.
[16] M.B. Mansur, M. Emerenciano, L. Brewer, M. Sant'Ana, N. Mendonca, L.C. Thuler, S. Koifman, M.S. Pombo-de-Oliveira, SIL-TAL1 fusion gene negative impact in T-cell acute lymphoblastic leukemia outcome, Leuk. Lymphoma 50 (2009) 1318–1325.
[17] M. Emerenciano, J. Menezes, M.L. Vasquez, I. Zalcberg, L.C. Thuler, M.S. Pombo-de-Oliveira, Clinical relevance of FLT3 gene abnormalities in Brazilian patients with infant leukemia, Leuk. Lymphoma 49 (2008) 2291–2297.
[18] M. Nakao, S. Yokota, T. Iwai, H. Kaneko, S. Horiike, K. Kashima, Y. Sonoda, T. Fujimoto, S. Misawa, Internal tandem duplication of the flt3 gene found in acute myeloid leukemia, Leukemia 10 (1996) 1911–1918.
[19] P.P. Zenatti, D. Ribeiro, W. Li, L. Zuurbier, M.C. Silva, M. Paganin, J. Tritapoe, J.A. Hixon, A.B. Silveira, B.A. Cardoso, L.M. Sarmento, N. Correia, M.L. Toribio, J. Kobarg, M. Horstmann, R. Pieters, S.R. Brandalise, A.A. Ferrando, J.P. Meijerink, S.K. Durum, J.A. Yunes, J.T. Barata, Oncogenic IL7R gain-of-function mutations in childhood T-cell acute lymphoblastic leukemia, Nat. Genet. 43 (2011) 932–939.
[20] O. Hrusak, A. Porwit-MacDonald, Antigen expression patterns reflecting genotype of acute leukemias, Leukemia 16 (2002) 1233–1258.
[21] M. van Grotel, J.P. Meijerink, E.R. van Wering, A.W. Langerak, H.B. Beverloo, J.G. Buijs-Gladdines, N.B. Burger, M. Passier, E.M. van Lieshout, W.A. Kamps, A.J. Veerman, M.M. van Noesel, R. Pieters, Prognostic significance of molecular-cytogenetic abnormalities in pediatric T-ALL is not explained by immunophenotypic differences, Leukemia 22 (2008) 124–131.
[22] E. Coustan-Smith, C.G. Mullighan, M. Onciu, F.G. Behm, S.C. Raimondi, D. Pei, C. Cheng, X. Su, J.E. Rubnitz, G. Basso, A. Biondi, C.H. Pui, J.R. Downing, D. Campana, Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia, Lancet Oncol. 10 (2009) 147–156.
[23] T. Inukai, N. Kiyokawa, D. Campana, E. Coustan-Smith, A. Kikuchi, M. Kobayashi, H. Takahashi, K. Koh, A. Manabe, M. Kumagai, K. Ikuta, Y. Hayashi, M. Tsuchida, K. Sugita, A. Ohara, Clinical significance of early T-cell precursor acute lymphoblastic leukaemia: results of the Tokyo Children's Cancer Study Group Study L99-15, Br. J. Haematol. 156 (2012) 358–365.
[24] E. Paietta, A.A. Ferrando, D. Neuberg, J.M. Bennett, J. Racevskis, H. Lazarus, G. Dewald, J.M. Rowe, P.H. Wiernik, M.S. Tallman, A.T. Look, Activating FLT3 mutations in CD117/KIT(+) T-cell acute lymphoblastic leukemias, Blood 104 (2004) 558–560.
[25] H.G. Drexler, Expression of FLT3 receptor and response to FLT3 ligand by leukemic cells, Leukemia 10 (1996) 588–599.
[26] E.A. Macintyre, L. Smit, J. Ritz, I.R. Kirsch, J.L. Strominger, Disruption of the SCL locus in T-lymphoid malignancies correlates with commitment to the T-cell receptor alpha beta lineage, Blood 80 (1992) 1511–1520.
[27] M. Neumann, S. Heesch, N. Gokbuget, S. Schwartz, C. Schlee, O. Benlasfer, N. Farhadi-Sartangi, J. Thibaut, T. Burmeister, D. Hoelzer, W.K. Hofmann, E. Thiel, C.D. Baldus, Clinical and molecular characterization of early T-cell precursor leukemia: a high-risk subgroup in adult T-ALL with a high frequency of FLT3 mutations, Blood Cancer J. 2 (2012), e55.
[28] J. Zhang, L. Ding, L. Holmfeldt, G. Wu, S.L. Heatley, D. Payne-Turner, J. Easton, X. Chen, J. Wang, M. Rusch, C. Lu, S.C. Chen, L. Wei, J.R. Collins-Underwood, J. Ma, K.G. Roberts, S.B. Pounds, A. Ulyanov, J. Becksfort, P. Gupta, R. Huether, R.W. Kriwacki, M. Parker, D.J. McGoldrick, D. Zhao, D. Alford, S. Espy, K.C. Bobba, G. Song, D. Pei, C. Cheng, S. Roberts, M.I. Barbato, D. Campana, E. Coustan-Smith, S.A. Shurtleff, S.C. Raimondi, M. Kleppe, J. Cools, K.A. Shimano, M.L. Hermiston, S. Doulatov, K. Eppert, E. Laurenti, F. Notta, J.E. Dick, G. Basso, S.P. Hunger, M.L. Loh, M. Devidas, B. Wood, S. Winter, K.P. Dunsmore, R.S. Fulton, L.L. Fulton, X. Hong, C.C. Harris, D.J. Dooling, K. Ochoa, K.J. Johnson, J.C. Obenauer, W.E. Evans, C.H. Pui, C.W. Naeve, T.J. Ley, E.R. Mardis, R.K. Wilson, J.R. Downing, C.G. Mullighan, The genetic basis of early T-cell precursor acute lymphoblastic leukaemia, Nature 481 (2012) 157–163.
[29] M. Neumann, E. Coskun, L. Fransecky, L.H. Mochmann, I. Bartram, N.F. Sartangi, S. Heesch, N. Gokbuget, S. Schwartz, C. Brandts, C. Schlee, R. Haas, U. Duhrsen, M. Griesshammer, H. Dohner, G. Ehninger, T. Burmeister, O. Blau, E. Thiel, D. Hoelzer, W.K. Hofmann, C.D. Baldus, FLT3 mutations in early T-cell precursor ALL characterize a stem cell like leukemia and imply the clinical use of tyrosine kinase inhibitors, PLoS One 8 (2013), e53190.
[30] C.M. Zaremba, D. Oliver, M. Cavalier, F. Fuda, N.J. Karandikar, W. Chen, Distinct immunophenotype of early T-cell progenitors in T lymphoblastic leukemia/lymphoma may predict FMS-like tyrosine kinase 3 mutations, Ann. Diagn. Pathol. 16 (2012) 16–20.
[31] D. Hoehn, L.J. Medeiros, S.S. Chen, T. Tian, J.L. Jorgensen, Y. Ahmed, P. Lin, CD117 expression is a sensitive but nonspecific predictor of FLT3 mutation in T acute lymphoblastic leukemia and T/myeloid acute leukemia, Am. J. Clin. Pathol. 137 (2012) 213–219.
[32] C. Sperling, S. Schwartz, T. Buchner, E. Thiel, W.D. Ludwig, Expression of the stem cell factor receptor C-KIT (CD117) in acute leukemias, Haematologica 82 (1997) 617–621.
[33] M.C. Bene, M. Bernier, R.O. Casasnovas, G. Castoldi, W. Knapp, F. Lanza, W.D. Ludwig, E. Matutes, A. Orfao, C. Sperling, M.B. van't Veer, The reliability and specificity of c-kit for the diagnosis of acute myeloid leukemias and undifferentiated leukemias. The European Group for the Immunological Classification of Leukemias (EGIL), Blood 92 (1998) 596–599.
[34] P. Van Vlierberghe, A. Ambesi-Impiombato, K. De Keersmaecker, M. Hadler, E. Paietta, M.S. Tallman, J.M. Rowe, C. Forne, M. Rue, A.A. Ferrando, Prognostic relevance of integrated genetic profiling in adult T-cell acute lymphoblastic leukemia, Blood 122 (2013) 74–82.
[35] T. Jahn, S. Sindhu, S. Gooch, P. Seipel, P. Lavori, E. Leifheit, K. Weinberg, Direct interaction between Kit and the interleukin-7 receptor, Blood 110 (2007) 1840–1847.